FIGURE 1: SEQ ID NO:1

Nucleotide Sequence Tankyrase homologue isotype1

```
CTTTGAAGACACTGGATTTCATACTTTTGCCTGGGGTTATCTCTCTGTGTCTCACTACATAGACAAATA
TTAGCTGTGAGCAGATCTTTTTTTGTTGCTTCTTGTAGTCCCCCAGTTTAGCAGAAACATTCTGTGAGA
TAGATGTGGGAAAGGAATTCTAGCAAGAGTTTTGTCACTGTATCATAAGGTTGTGATTTACATATTTAA
GTTTTATACTTTGAACATCTGAAAATGTATACATACTAAATATGCAGAACTCTATTGTAGAGTGAGAAA
CATTTGAACTTTGAGCTTTCAGTCACTTATTTTGTATTCTTTCTTTGAGGTTAGCAGTAGTACCACCCA
AGGCACTGCTTAGGTACCACTGCTGCTTAGTGGAGAGTCCCTCTGGCTTTATCATTAAGGTTTTGGGCG
GAAAGACGTAGTTGAATATTTGCTTCAGAATGGTGCAAGTGTCCAAGCACGTGATGATGGGGGCCTTAT
TCCTCTTCATAATGCATGCTCTTTTGGTCATGCTGAAGTAGTCAATCTCCTTTTGCGACATGGTGCAGA
CCCCAATGCTCGAGATAATTGGAATTATACTCCTCTCCATGAAGCTGCAATTAAAGGAAAGATTGATGT
TTGCATTGTGCTGTTACAGCATGGAGCTGAGCCAACCATCCGAAATACAGATGGAAGGACAGCATTGGA
TTTAGCAGATCCATCTGCCAAAGCAGTGCTTACTGGTGAATATAAGAAAGATGAACTCTTAGAAAGTGC
CAGGAGTGGCAATGAAGAAAAAATGATGGCTCTACTCACACCATTAAATGTCAACTGCCACGCAAGTGA
TGGCAGAAAGTCAACTCCATTACATTTGGCAGCAGGATATAACAGAGTAAAGATTGTACAGCTGTTACT
GCAACATGGAGCTGATGTCCATGCTAAAGATAAAGGTGATCTGGTACCATTACACAATGCCTGTTCTTA
TGGTCATTATGAAGTAACTGAACTTTTGGTCAAGCATGGTGCCTGTGTAAATGCAATGGACTTGTGGCA
ATTCACTCCTCTTCATGAGGCAGCTTCTAAGAACAGGGTTGAAGTATGTTCTCTTCTCTTAAGTTATGG
TGCAGACCCAACACTGCTCAATTGTCACAATAAAAGTGCTATAGACTTGGCTCCCACACCACAGTTAAA
AGAAAGATTAGCATATGAATTTAAAGGCCACTCGTTGCTGCAAGCTGCACGAGAAGCTGATGTTACTCG
AATCAAAAAACATCTCTCTCTGGAAATGGTGAATTTCAAGCATCCTCAAACACATGAAACAGCATTGCA
TTGTGCTGCTGCATCTCCATATCCCAAAAGAAAGCAAATATGTGAACTGTTGCTAAGAAAAGGAGCAAA
CATCAATGAAAAGACTAAAGAATTCTTGACTCCTCTGCACGTGGCATCTGAGAAAGCTCATAATGATGT
TGTTGAAGTAGTGGTGAAACATGAAGCAAAGGTTAATGCTCTGGATAATCTTGGTCAGACTTCTCTACA
CAGAGCTGCATATTGTGGTCATCTACAAACCTGCCGCCTACTCCTGAGCTATGGGTGTGATCCTAACAT
TATATCCCTTCAGGGCTTTACTGCTTTACAGATGGGAAATGAAAATGTACAGCAACTCCTCCAAGAGGG
TATCTCATTAGGTAATTCAGAGGCAGACAGACAATTGCTGGAAGCTGCAAAGGCTGGAGATGTCGAAAC
TGTAAAAAAACTGTGTACTGTTCAGAGTGTCAACTGCAGAGACATTGAAGGGCGTCAGTCTACACCACT
TCATTTTGCAGCTGGGTATAACAGAGTGTCCGTGGTGGAATATCTGCTACAGCATGGAGCTGATGTGCA
TGCTAAAGATAAAGGAGGCCTTGTACCTTTGCACAATGCATGTTCTTATGGACATTATGAAGTTGCAGA
ACTTCTTGTTAAACATGGAGCAGTAGTTAATGTAGCTGATTTATGGAAATTTACACCTTTACATGAAGC
AGCAGCAAAAGGAAAATATGAAATTTGCAAACTTCTGCTCCAGCATGGTGCAGACCCTACCAAAAAAAA
CAGGGATGGAAATACTCCTTTGGATCTTGTTAAAGATGGAGATACAGATATTCAAGATCTGCTTAGGGG
AGATGCAGCTTTGCTAGATGCTGCCAAGAAGGGTTGTTTAGCCAGAGTGAAGAAGTTGTCTTCTCCTGA
TAATGTAAATTGCCGCGATACCCAAGGCAGACATTCAACACCTTTACATTTAGCAGCTGGTTATAATAA
TTTAGAAGTTGCAGAGTATTTGTTACAACACGGAGCTGATGTGAATGCCCAAGACAAAGGAGGACTTAT
TCCTTTACATAATGCAGCATCTTACGGGCATGTAGATGTAGCAGCTCTACTAATAAAGTATAATGCATG
TGTCAATGCCACGGACAAATGGGCTTTCACACCTTTGCACGAAGCAGCCCAAAAGGGACGAACACAGCT
TTGTGCTTTGTTGCTAGCCCATGGAGCTGACCCGACTCTTAAAAATCAGGAAGGACAAACACCTTTAGA
TTTAGTTTCAGCGGATGATGTCAGCGCTCTTCTGACAGCAGCCATGCCCCCATCTGCTCTGCCCTCTTG
TTACAAGCCTCAAGTGCTCAATGGTGTGAGAAGCCCAGGAGCCACTGCAGATGCTCTCTCTTCAGGTCC
ATCTAGCCCATCAAGCCTTTCTGCAGCCAGCAGTCTTGACAACTTATCTGGGAGTTTTTTCAGAACTGTC
TTCAGTAGTTAGTTCAAGTGGAACAGAGGGTGCTTCCAGTTTGGAGAAAAAGGAGGTTCCAGGAGTAGA
TTTTAGCATAACTCAATTCGTAAGGAATCTTGGACTTGAGCACCTAATGGATATATTTGAGAGAGAACA
GATCACTTTGGATGTATTAGTTGAGATGGGGCACAAGGAGCTGAAGGAGATTGGAATCAATGCTTATGG
ACATAGGCACAAACTAATTAAAGGAGTCGAGAGACTTATCTCCGGACAACAAGGTCTTAACCCATATTT
AACTTTGAACACCTCTGGTAGTGGAACAATTCTTATAGATCTGTCTCCTGATGATAAAGAGTTTCAGTC
TGTGGAGGAAGAGATGCAAAGTACAGTTCGAGAGCACAGAGATGGAGGTCATGCAGGTGGAATCTTCAA
CAGATACAATATTCTCAAGATTCAGAAGGTTTGTAACAAGAAACTATGGGAAAGATACACTCACCGGAG
AAAAGAAGTTTCTGAAGAAAACCACAACCATGCCAATGAACGAATGCTATTTCATGGGTCTCCTTTTGT
GAATGCAATTATCCACAAAGGCTTTGATGAAAGGCATGCGTACATAGGTGGTATGTTTGGAGCTGGCAT
TTATTTTGCTGAAAACTCTTCCAAAAGCAATCAATATGTATATGGAATTGGAGGAGGTACTGGGTGTCC
AGTTCACAAAGACAGATCTTGTTACATTTGCCACAGGCAGCTGCTCTTTTGCCGGGTAACCTTGGGAAA
GTCTTTCCTGCAGTTCAGTGCAATGAAAATGGCACATTCTCCTCCAGGTCATCACTCAGTCACTGGTAG
GCCCAGTGTAAATGGCCTAGCATTAGCTGAATATGTTATTTACAGAGGAGAACAGGCTTATCCTGAGTA
TTTAATTACTTACCAGATTATGAGGCCTGAAGGTATGGTCGATGGATAAATAGTTATTTTAAGAAACTA
ATTCCACTGAACCTAAAATCATCAAAGCAGCAGTGGCCTCTACGTTTTACTCCTTTGCTGAAAAAAAAA
AA
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68

FIGURE 2: SEQ ID NO:2

Nucleotide Sequence Tankyrase homologue isotype2

```
CGCGCTGCTCCGCCCGCCGCGGGGCAGCCGGGGGGCAGGGAGCCCAGCGAGGGGCGCGCGTGGGCGCGG
CCCATGGGACTGCGCCGGATCCGGTGACAGCAGGGAGCCAAGCGGCCCGGGCCCTGAGCGCGTCTTCTC
CGGGGGGGCCTCGCCCTCCTGCTCGCGGGGCCGGGGCTCCTGCTCCGGTTGCTGGCGCTGTTGCTGGCTG
TGGCGGCGGCCAGGATCATGTCGGGTCGCCGCTGCGCCGGCGGGGGAGCGGCCTGCGCGAGCGCCGCGG
CCGAGGCCGTGGAGCCGGCCGCCCGAGAGCTGTTCGAGGCGTGCCGCAACGGGGACGTGGAACGAGTCA
AGAGGCTGGTGACGCCTGAGAAGGTGAACAGCCGCGACACGGCGGGCAGGAAATCCACCCCGCTGCACT
TCGCCGCAGGTTTTGGGCGGAAAGACGTAGTTGAATATTTGCTTCAGAATGGTGCAAATGTCCAAGCAC
GTGATGATGGGGGCCTTATTCCTCTTCATAATGCATGCTCTTTTGGTCATGCTGAAGTAGTCAATCTCC
TTTTGCGACATGGTGCAGACCCCAATGCTCGAGATAATTGGAATTATACTCCTCTCCATGAAGCTGCAA
TTAAAGGAAAGATTGATGTTTGCATTGTGCTGTTACAGCATGGAGCTGAGCCAACCATCCGAAATACAG
ATGGAAGGACAGCATTGGATTTAGCAGATCCATCTGCCAAAGCAGTGCTTACTGGTGAATATAAGAAAG
ATGAACTCTTAGAAAGTGCCAGGAGTGGCAATGAAGAAAAAATGATGGCTCTACTCACACCATTAAATG
TCAACTGCCACGCAAGTGATGGCAGAAAGTCAACTCCATTACATTTGGCAGCAGGATATAACAGAGTAA
AGATTGTACAGCTGTTACTGCAACATGGAGCTGATGTCCATGCTAAAGATAAAGGTGATCTGGTACCAT
TACACAATGCCTGTTCTTATGGTCATTATGAAGTAACTGAACTTTTGGTCAAGCATGGTGCCTGTGTAA
ATGCAATGGACTTGTGGCAATTCACTCCTCTTCATGAGGCAGCTTCTAAGAACAGGGTTGAAGTATGTT
CTCTTCTCTTAAGTTATGGTGCAGACCCAACACTGCTCAATTGTCACAATAAAAGTGCTATAGACTTGG
CTCCCACACCACAGTTAAAAGAAAGATTAGCATATGAATTTAAAGGCCACTCGTTGCTGCAAGCTGCAC
GAGAAGCTGATGTTACTCGAATCAAAAAACATCTCTCTCTGGAAATGGTGAATTTCAAGCATCCTCAAA
CACATGAAACAGCATTGCATTGTGCTGCTGCATCTCCATATCCCAAAAGAAAGCAAATATGTGAACTGT
TGCTAAGAAAAGGAGCAAACATCAATGAAAAGACTAAAGAATTCTTGACTCCTCTGCACGTGGCATCTG
AGAAAGCTCATAATGATGTTGTTGAAGTAGTGGTGAAACATGAAGCAAAGGTTAATGCTCTGGATAATC
TTGGTCAGACTTCTCTACACAGAGCTGCATATTGTGGTCATCTACAAACCTGCCGCCTACTCCTGAGCT
ATGGGTGTGATCCTAACATTATATCCCTTCAGGGCTTTACTGCTTTACAGATGGGAAATGAAAATGTAC
AGCAACTCCTCCAAGAGGGTATCTCATTAGGTAATTCAGAGGCAGACAGACAATTGCTGGAAGCTGCAA
AGGCTGGAGATGTCGAAACTGTAAAAAAACTGTGTACTGTTCAGAGTGTCAACTGCAGAGACATTGAAG
GGCGTCAGTCTACACCACTTCATTTTGCAGCTGGGTATAACAGAGTGTCCGTGGTGGAATATCTGCTAC
AGCATGGAGCTGATGTGCATGCTAAAGATAAAGGAGGCCTTGTACCTTTGCACAATGCATGTTCTTATG
GACATTATGAAGTTGCAGAACTTCTTGTTAAACATGGAGCAGTAGTTAATGTAGCTGATTTATGGAAAT
TTACACCTTTACATGAAGCAGCAGCAAAAGGAAAATATGAAATTTGCAAACTTCTGCTCCAGCATGGTG
CAGACCCTACCAAAAAAAACAGGGATGGAAATACTCCTTTGGATCTTGTTAAAGATGGAGATACAGATA
TTCAAGATCTGCTTAGGGGAGATGCAGCTTTGCTAGATGCTGCCAAGAAGGGTTGTTTAGCCAGAGTGA
AGAAGTTGTCTTCTCCTGATAATGTAAATTGCCGCGATACCCAAGGCAGACATTCAACACCTTTACATT
TAGCAGCTGGTTATAATAATTTAGAAGTTGCAGAGTATTTGTTACAACACGGAGCTGATGTGAATGCCC
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TAATAAAGTATAATGCATGTGTCAATGCCACGGACAAATGGGCTTTCACACCTTTGCACGAAGCAGCCC
AAAAGGGACGAACACAGCTTTGTGCTTTGTTGCTAGCCCATGGAGCTGACCCGACTCTTAAAAATCAGG
AAGGACAAACACCTTTAGATTTAGTTTCAGCGGATGATGTCAGCGCTCTTCTGACAGCAGCCATGCCCC
CATCTGCTCTGCCCTCTTGTTACAAGCCTCAAGTGCTCAATGGTGTGAGAAGCCCAGGAGCCACTGCAG
ATGCTCTCTCTTCAGGTCCATCTAGCCCATCAAGCCTTTCTGCAGCCAGCAGTCTTGACAACTTATCTG
GGAGTTTTTCAGAACTGTCTTCAGTAGTTAGTTCAAGTGGAACAGAGGGTGCTTCCAGTTTGGAGAAAA
AGGAGGTTCCAGGAGTAGATTTTAGCATAACTCAATTCGTAAGGAATCTTGGACTTGAGCACCTAATGG
ATATATTTGAGAGAGAACAGATCACTTTGGATGTATTAGTTGAGATGGGGCACAAGGAGCTGAAGGAGA
TTGGAATCAATGCTTATGGACATAGGCACAAACTAATTAAAAGGAGTCGAGAGACTTATCTCCGGACAAC
AAGGTCTTAACCCATATTTAACTTTGAACACCTCTGGTAGTGGAACAATTCTTATAGATCTGTCTCCTG
ATGATAAAGAGTTTCAGTCTGTGGAGGAAGAGATGCAAAGTACAGTTCGAGAGCACAGAGATGGAGGTC
ATGCAGGTGGAATCTTCAACAGATACAATATTCTCAAGATTCAGAAGGTTTGTAACAAGAAACTATGGG
AAAGATACACTCACCGGAGAAAAGAAGTTTCTGAAGAAAACCACAACCATGCCAATGAACGAATGCTAT
TTCATGGGTCTCCTTTTGTGAATGCAATTATCCACAAAGGCTTTGATGAAAGGCATGCGTACATAGGTG
GTATGTTTGGAGCTGGCATTTATTTTGCTGAAAACTCTTCCAAAAGCAATCAATATGTATATGGAATTG
GAGGAGGTACTGGGTGTCCAGTTCACAAAGACAGATCTTGTTACATTTGCCACAGGCAGCTGCTCTTTT
GCCGGGTAACCTTGGGAAAGTCTTTCCTGCAGTTCAGTGCAATGAAAATGGCACATTCTCCTCCAGGTC
ATCACTCAGTCACTGGTAGGCCCAGTGTAAATGGCCTAGCATTAGCTGAATATGTTATTTACAGAGGAG
AACAGGCTTATCCTGAGTATTTAATTACTTACCAGATTATGAGGCCTGAAGGTATGGTCGATGGATAAA
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69

TAGTTATTTTAAGAAACTAATTCCACTGAACCTAAAATCATCAAAGCAGCAGTGGCCTCTACGTTTTAC
TCCTTTGCTGAAAAAAAAAA

FIGURE 3: SEQ ID NO:3

Amino Acid Sequence Tankyrase homologue isotype1

GFGRKDVVEYLLQNGASVQARDDGGLIPLHNACSFGHAEVVNLLLRHGADPNARDNWNYTPLHEAAIKG
KIDVCIVLLQHGAEPTIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSGNEEK**MM**ALLTPLNVNC
HASDGRKSTPLHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVTELLVKHGACVNAM
DLWQFTPLHEAASKNRVEVCSLLLSYGADPTLLNCHNKSAIDLAPTPQLKERLAYEFKGHSLLQAAREA
DVTRIKKHLSLEMVNFKHPQTHETALHCAAASPYPKRKQICELLLRKGANINEKTKEFLTPLHVASEKA
HNDVVEVVVKHEAKVNALDNLGQTSLHRAAYCGHLQTCRLLLSYGCDPNIISLQGFTALQMGNENVQQL
LQEGISLGNSEADRQLLEAAKAGDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVVEYLLQHG
ADVHAKDKGGLVPLHNACSYGHYEVAELLVKHGAVVNVADLWKFTPLHEAAAKGKYEICKLLLQHGADP
TKKNRDGNTPLDLVKDGDTDIQDLLRGDAALLDAAKKGCLARVKKLSSPDNVNCRDTQGRHSTPLHLAA
GYNNLEVAEYLLQHGADVNAQDKGGLIPLHNAASYGHVDVAALLIKYNACVNATDKWAFTPLHEAAQKG
RTQLCALLLAHGADPTLKNQEGQTPLDLVSADDVSALLTAAMPPSALPSCYKPQVLNGVRSPGATADAL
SSGPSSPSSLSAASSLDNLSGSFSELSSVVSSSGTEGASSLEKKEVPGVDFSITQFVRNLGLEHLMDIF
EREQITLDVLVEMGHKELKEIGINAYGHRHKLIKGVERLISGQQGLNPYLTLNTSGSGTILIDLSPDDK
EFQSVEEEMQSTVREHRDGGHAGGIFNRYNILKIQKVCNKKLWERYTHRRKEVSEENHNHANERMLFHG
SPFVNAIIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPVHKDRSCYICHRQLLFCRV
TLGKSFLQFSAMKMAHSPPGHHSVTGRPSV

Bold = potential starting methionine

FIGURE 4: SEQ ID NO:4

Amino Acid Sequence Tankyrase homologue isotype2

RCSARRGAAGGQGAQRGARVGAAHGTAPDPVTAGSQAARALSASSPGGLALLLAGPGLLLRLLALLLAV
AAARIMSGRRCAGGGAACASAAAEAVEPAARELFEACRNGDVERVKRLVTPEKVNSRDTAGRKSTPLHF
AAGFGRKDVVEYLLQNGANVQARDDGGLIPLHNACSFGHAEVVNLLLRHGADPNARDNWNYTPLHEAAI
KGKIDVCIVLLQHGAEPTIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSGNEEKMMALLTPLNV
NCHASDGRKSTPLHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVTELLVKHGACVN
AMDLWQFTPLHEAASKNRVEVCSLLLSYGADPTLLNCHNKSAIDLAPTPQLKERLAYEFKGHSLLQAAR
EADVTRIKKHLSLEMVNFKHPQTHETALHCAAASPYPKRKQICELLLRKGANINEKTKEFLTPLHVASE
KAHNDVVEVVVKHEAKVNALDNLGQTSLHRAAYCGHLQTCRLLLSYGCDPNIISLQGFTALQMGNENVQ
QLLQEGISLGNSEADRQLLEAAKAGDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVVEYLLQ
HGADVHAKDKGGLVPLHNACSYGHYEVAELLVKHGAVVNVADLWKFTPLHEAAAKGKYEICKLLLQHGA
DPTKKNRDGNTPLDLVKDGDTDIQDLLRGDAALLDAAKKGCLARVKKLSSPDNVNCRDTQGRHSTPLHL
AAGYNNLEVAEYLLQHGADVNAQDKGGLIPLHNAASYGHVDVAALLIKYNACVNATDKWAFTPLHEAAQ
KGRTQLCALLLAHGADPTLKNQEGQTPLDLVSADDVSALLTAAMPPSALPSCYKPQVLNGVRSPGATAD
ALSSGPSSPSSLSAASSLDNLSGSFSELSSVVSSSGTEGASSLEKKEVPGVDFSITQFVRNLGLEHLMD
IFEREQITLDVLVEMGHKELKEIGINAYGHRHKLIKGVERLISGQQGLNPYLTLNTSGSGTILIDLSPD
DKEFQSVEEEMQSTVREHRDGGHAGGIFNRYNILKIQKVCNKKLWERYTHRRKEVSEENHNHANERMLF
HGSPFVNAIIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPVHKDRSCYICHRQLLFC
RVTLGKSFLQFSAMKMAHSPPGHHSVTGRPSVNGLALAEYVIYRGEQAYPEYLITYQIMRPEGMVDG

70

FIGURE 5

Schematic Presentation of Dominant negative Mutants for Tankyrase Homologue

Dominant Negative Mutants:

Truncation: 429ΔC- of the C-terminal catalytic domain – truncation of the catalytic domain of PARP acts as a dominant negative when overexpressed *in vivo* (Oncogene 1999 Nov 25;18(50):7010-5)

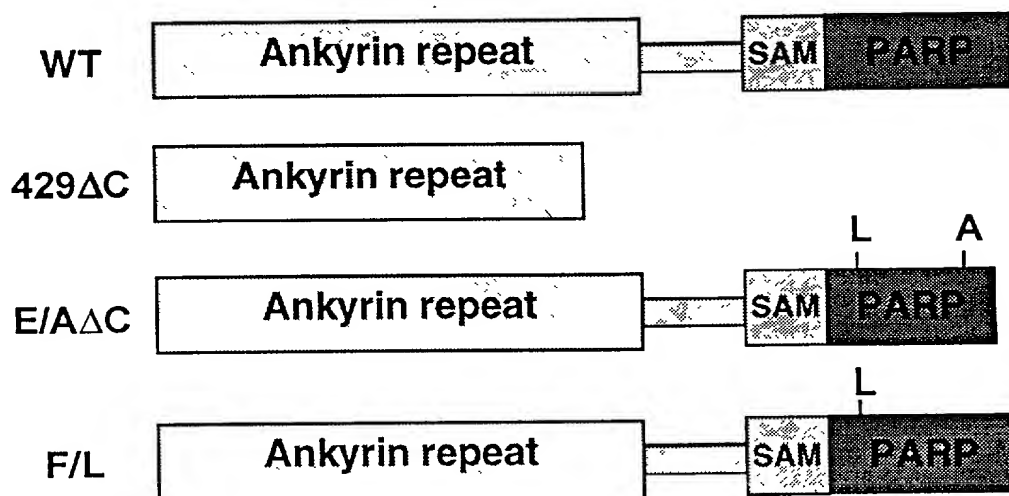Point mutant: E945AΔC- conserved residue in PARP domain, thought to be important in NAD+ binding

WT   Ankyrin repeat   SAM   PARP

429ΔC   Ankyrin repeat

E/AΔC   Ankyrin repeat   SAM   PARP   L   A

F/L   Ankyrin repeat   SAM   PARP   L

7]

FIGURE 6

Cell Cycle Analysis of A549 Cells Infected With GFP-fused Wild Type and Mutant Tankyrase Homologue

Mock
WT
F/L
E/A ΔC
429 ΔC
p21

Relative Cell Number

Relative Cell Number

GFP Fluorescent Intensity

Hoescht Staining

Gates for GFP positive cells and negative cells are shown in FACS analysis of GFP expression

Cells were infected retrovirus encoding GFP-fused wild type and mutant Tankyrase homologue and incubated for 48 hours.

—— Cell cycle analysis of GFP positive cells

········· Cell cycle analysis of GFP negative cells

72

FIGURE 7

Kinetics of GFP Positive cells in A549 Cells and Human Mammary Epithelial Cells(HMEC) After Retrovirus Infection Encoding GFP-fused Wild Type and Mutant Tankyrase Homologue
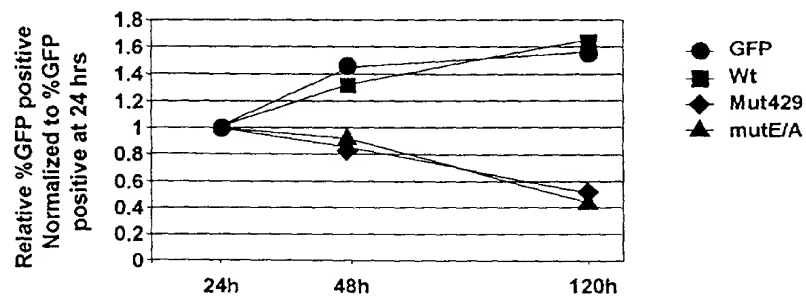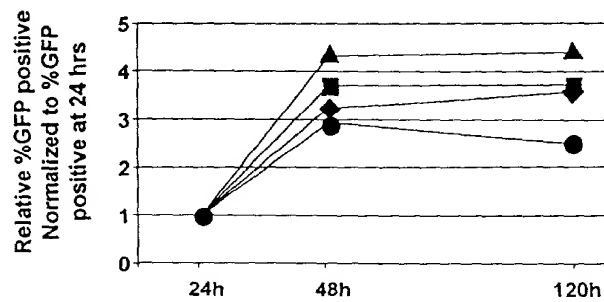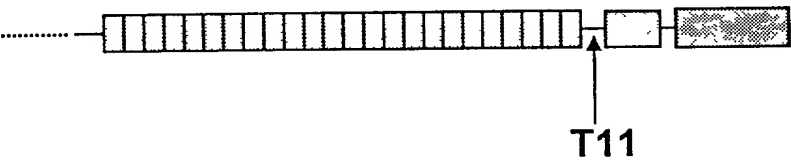
FIGURE 8

The Binding Site of Antisense Oligos Against Tankyrase Homologue

Tankyrase Homologue

T11

☐ Ankyrin repeat   ▨ SAM   ▨ PARP

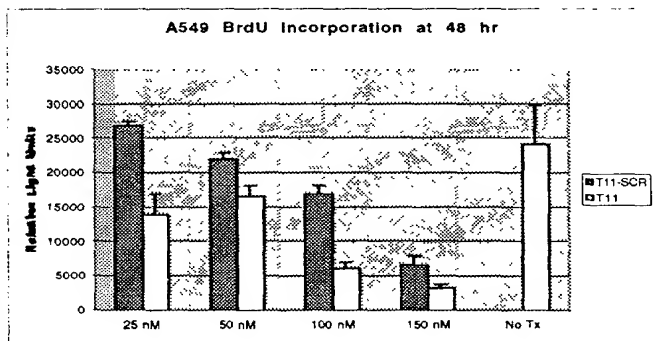········· Unidentified sequence

T11

GTGGAACAGAGGGTGCTTCC

```
Tankyrase Homologue  GTGGAACAGAGGGTGCTTCCAGTTTGGAGAAAAAGGAGGTTCCAGGAGTAGATTTTAGCAT  2838
Tankyrase            ATGCAGGGGATGGCGCCGCGGGAACAGAAAGGAAGGAAGGAGAAGTTGCTGGTCTTGACAT  3091
                        * *  *   ** ** **  *  *     ** *  ***** *     **  *  *  * ** ***
```

FIGURE 9

Anti-Proliferative Phenotype of Antisense Oligonucleotides Against Tankyrase Homologue in A549 and HeLa Cells
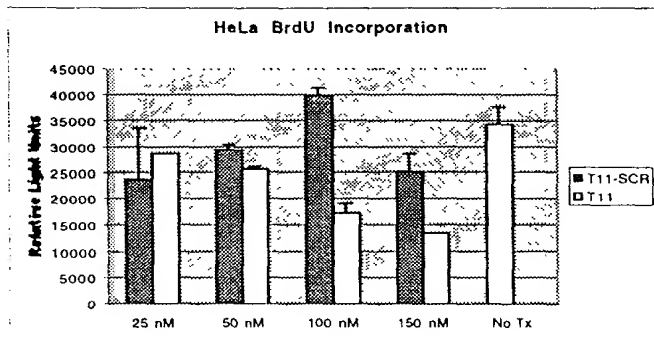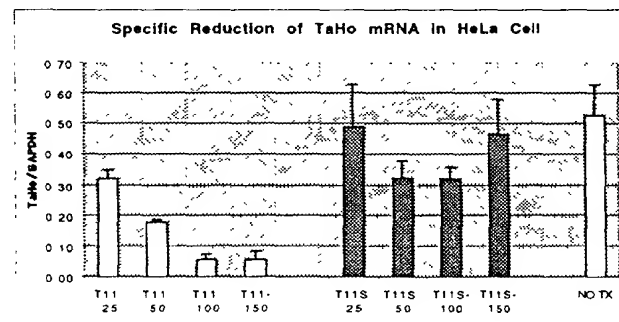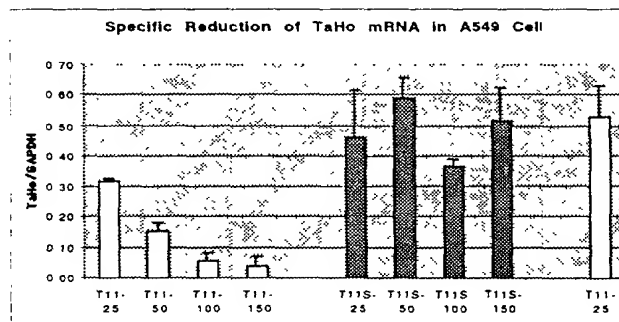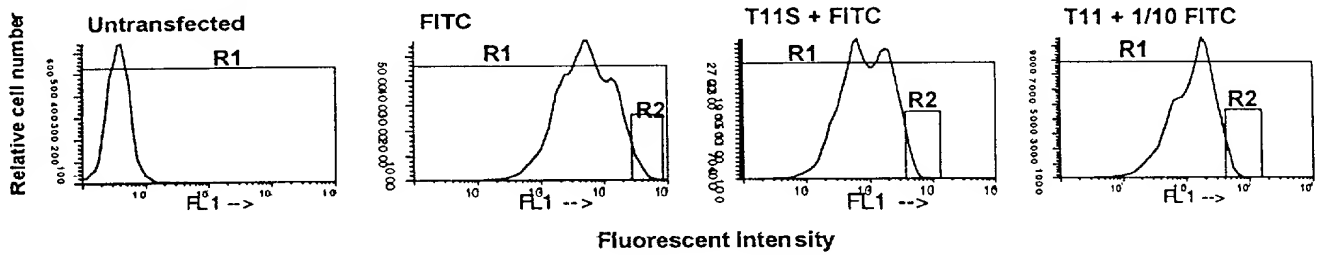
FIGURE 10

Cell Cycle Analysis of A549 Cells Transfected with Antisense Oligonucleotides Against Tankyrase Homologue at 48 Hours, Antisense Oligonucleotides (T11) and control oligonucleotides (T11S) were transfected with FITC-labeled random 20mer oligonucleotides(FITC), After 48 hours, entire population(R1) and top 5 % (R2)of FITC transfected cells were analyzed for cell cycle.

A) Gates for cell cycle analysis

Fluorescent Intensity

B) Cell cycle analysis

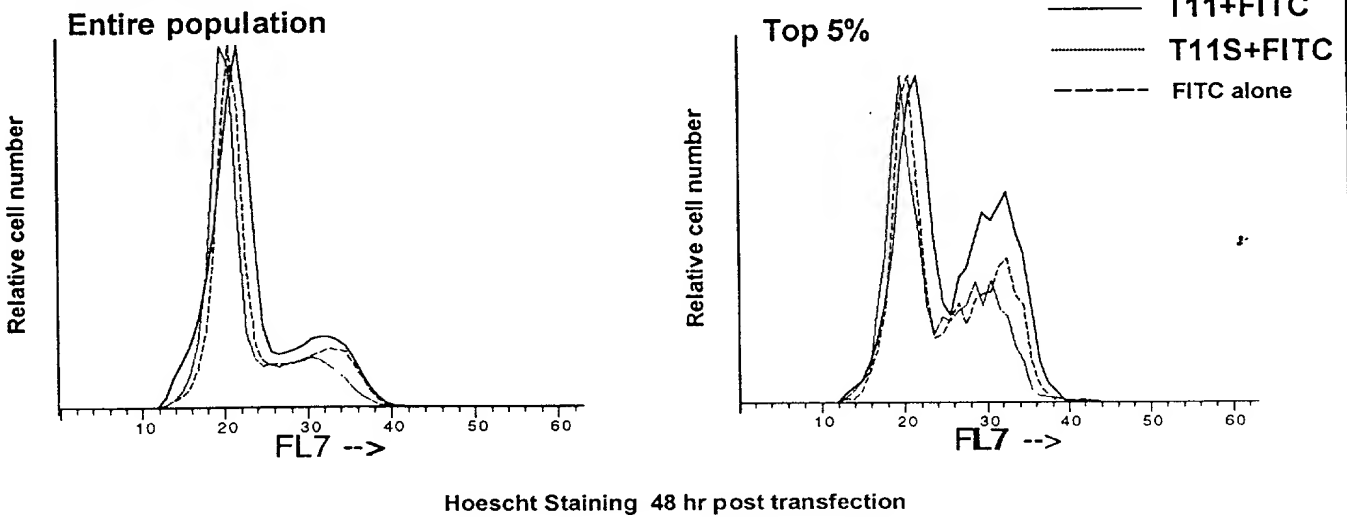Hoescht Staining 48 hr post transfection

FIGURE 11

mRNA expression of Tankyrase Homologue in Several Tumors and Normal Tissues by a Taqman Analysis, mRNA expression was normalized by 90kDa Highly Basic Protien (HBP) and ribosomal protein S9 (S9).
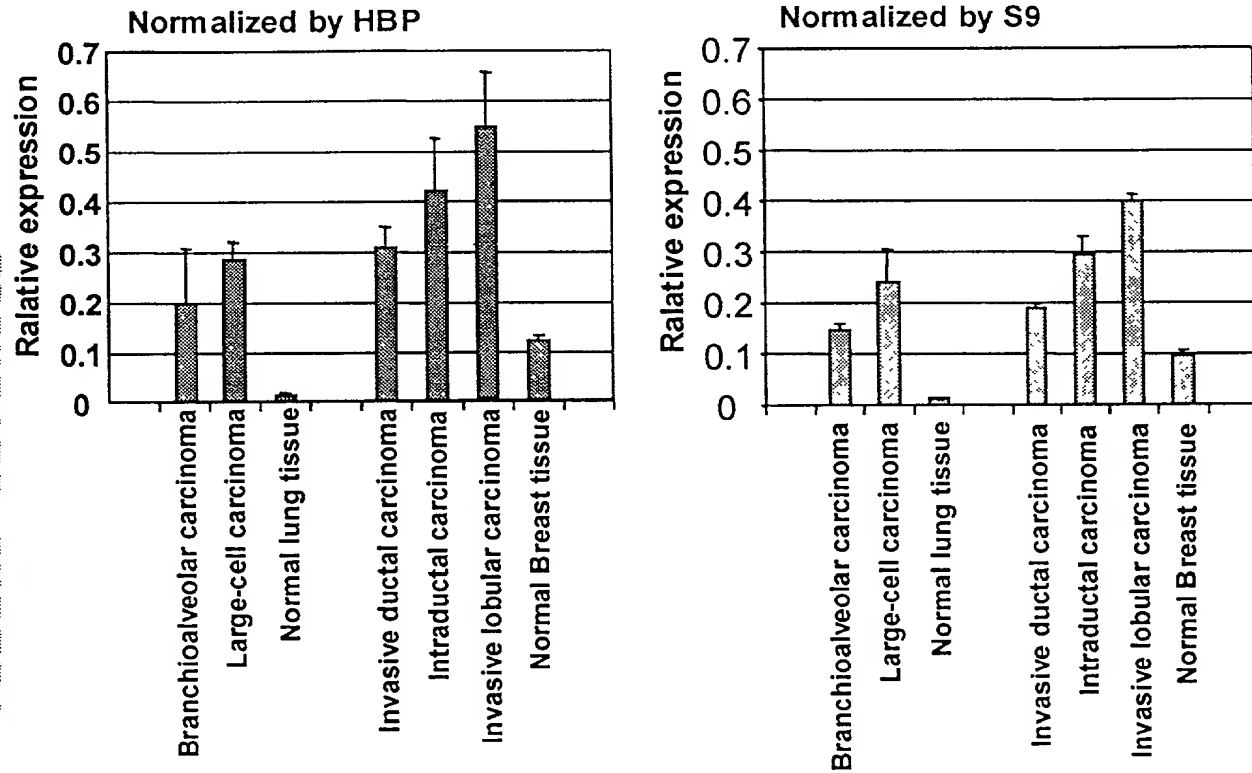
FIGURE 12

Procedure for Nonisotopic Detection of Poly-ADP Ribosylation Using Anti-GFP mAb-Coated Plates

Protein lysates from 293T cells normalized by GFP fluorescence and total protein

↓

Immobilization of GFP-tankyrase homologue in anti-GFP Coated plates

↓

Auto PARP reaction with Biotinylated-NAD in 96 wells

↓

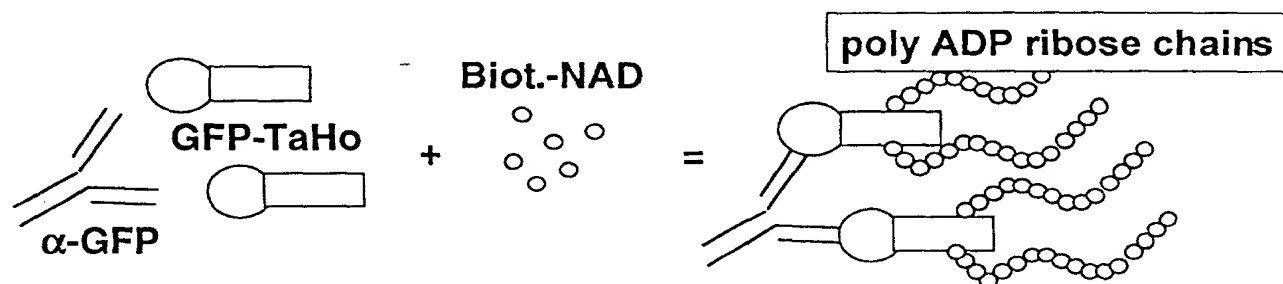Detection of poly ADP ribose chains with Streptavidin-HRP and chemiluminescent substrate

GFP-TaHo   +   Biot.-NAD   =   poly ADP ribose chains

α-GFP

FIGURE 13

Non-Isotopic Plate-Based Detection of Taho PARP Activity in the Presence of Biotinylated NAD.
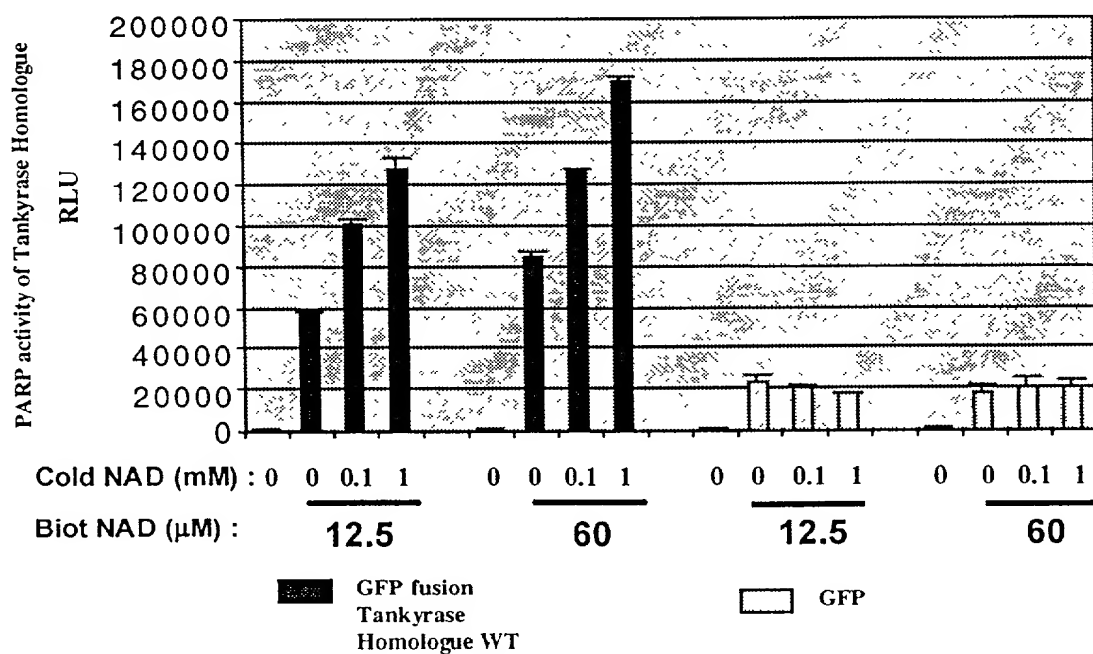
FIGURE 14

Comparison of IC$_{50}$ Values of the PARP Inhibitors

	Approximate IC$_{50}$ (nM)	hPARP assay IC$_{50}$ (nM)		
	TaHo	Rigel	Decker *	Rankin *
3AB	> 50 000	5 000	2 000	5 400
6(5H)Phenanthridinone	1 000-2 000	300		
Niacinamide	> 50 000	30 000	>>5 000	31 000

* Decker P et al., Clinical Cancer Research. 1999 May; 5:1169-1172.
* Rankin PW et al., J Biol Chem. 1989 Mar 15;264(8):4312-4317.

80

FIGURE 15

Inhibition of Tankyrase homologue PARP activity by hPARP inhibitors

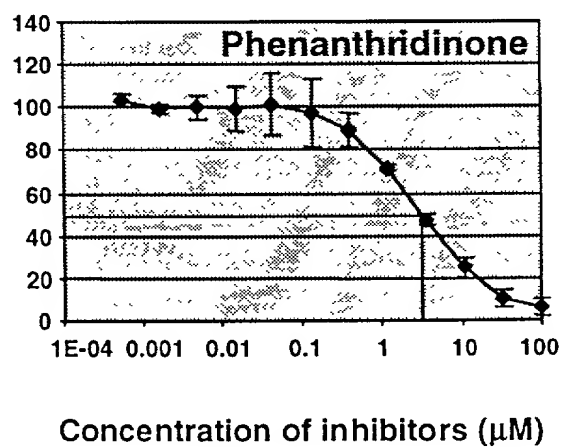Concentration of inhibitors (µM)

FIGURE 16
(sheet 1 of 3)

Legend for FIGURE 16

TH-1: Tankyrase homologue isoform-1, TH-2: Tankyrase homologue isoform-2
M (Red): the first methionine in the sequence, Z: stop codon
In this figure, the first methyonine in TH-1 sequence is position 1 (M1)

Taho C terminus deletion mutant ends at position 429 (K) and adds 28 amino acids because of frame shift.

Taho F/L mutant has the mutation at position 871

Taho E/A dC mutant has the mutation at position 948, ends at position 957 (A) and adds 2 amino acids.

```
TH-1    --------------------------------------------------------
TH-2    ---------------------RCSARRGAAGGQGAQRGARVGAAHGTAPDPVTAGSQ  -231

TH-1    --------------------------------------------------------
TH-2    AARALSASSPGGLALLLAGPGLLLRLLALLLAVAAARIMSGRRCAGGGAACASAAAEAVE  -171

TH-1    -----------*GFGRKDVVEYLLQNGA  -111
TH-2    PAARELFEACRNGDVERVKRLVTPEKVNSRDTAGRKSTPLHFAAGFGRKDVVEYLLQNGA  -111
                          Ankyrin repeat

TH-1    SVQARDDGGLIPLHNACSFGHAEVVNLLLRHGADPNARDNWNYTPLHEAAIKGKIDVCIV  -51
TH-2    NVQARDDGGLIPLHNACSFGHAEVVNLLLRHGADPNARDNWNYTPLHEAAIKGKIDVCIV  -51
               Ankyrin repeat              Ankyrin repeat
                                            •TH1 start
TH-1    LLQHGAEPTIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSGNEEKMALLTPLNV  10
TH-2    LLQHGAEPTIRNTDGRTALDLADPSAKAVLTGEYKKDELLESARSGNEEKMALLTPLNV  10
```

FIGURE 16
(sheet 2 of 3)

	Ankyrin repeat

TH-1 NCHASDGRKSTPLHLAAGYNRVKIVQLLQHGADVHAKDKGDLVPLHNACSYGHYEVTEL 70
TH-2 NCHASDGRKSTPLHLAAGYNRVKIVQLLQHGADVHAKDKGDLVPLHNACSYGHYEVTEL 70
          Ankyrin repeat

TH-1 LVKHGACVNAMDLWQFTPLHEAASKNRVEVCSLLLSYGADPTLLNCHNKSAIDLAPTPQL 130
TH-2 LV
              Ankyrin repeat

TH-1 KERLAYEFKGHSLLQAAREADVTRIKKHLSLEMVNFKHPQTHETALHCAAASPYPKRKQI 190
          Ankyrin repeat

TH-1 CELLLRKGANINEKTKEFLTPLHVASEKAHNDVVEVVVKHEAKVNALDNLGQTSLHRAAY 250
                Ankyrin repeat

TH-1 CGHLQTCRLLLSYGCDPNIISLQGFTALQMGNENVQQLLQEGISLGNSEADRQLLEAAKA 310
      Ankyrin repeat

TH-1 GDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVVEYLLQHGADVHAKDKGGLVP 370
      Ankyrin repeat

                                                              T
TH-1 LHNACSYGHYEVAELLVKHGAVVNVADLWKFTPLHEAAAKGKYEICKLLLQHGADPTKKN 430
                                                     Deletion---•
              Ankyrin repeat

TH-1 GMEILLWILLKMEIQIFKICLGEMQLCZ
      RDGNTPLDLVKDGDTDIQDLLRGDAALLDAAKKGCLARVKKLSSPDNVNCRDTQGRHSTP 490
          Ankyrin repeat

83

Figure image with sequence

FIGURE 16
(sheet 3 of 3)

TH-1 LHLAAGYNNLEVAEYLLQHGADVNAQDKGGLIPLHNAASYGHVDVAALLIKYNACVNATD 550
                        Ankyrin repeat

TH-1 KWAFTPLHEAAQKGRTQLCALLLAHGADPTLKNQEGQTPLDLVSADDVSALLTAAMPPSA 610
                        Ankyrin repeat

TH-1 LPSCYKPQVLNGVRSPGATADALSSGPSSPSSLSAASSLDNLSGSFSELSSVVSSSGTEG 670
                        Ankyrin repeat

TH-1 ASSLEKKE--VPGVDFSITQFVRNLGLEHLMDIFEREQITLDVLVEMGHKELKEIGINAY 730
                        SAM domain

TH-1 GHRHKLIKGVERLISGQQGLNPYLTLNTSGSGTILIDLSPDDKEFQSVEEEMQSTVREHR 790

TH-1 DGGHAGGIFNRYNILKIQKVCNKKLWERYTHRRKEVSEENHNHANERMLFHGSPFVNAII 850

TH-1 HKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPVHKDRSCYICHRQLLFCR 910
           • F→L mutation
           PARP domain

TH-1 VTLGKSFLQFSAMKMAHSPPGHHSVTGRPSVNGLALAEYVIYRGEQAYPEYLITYQIMRP 970
                    -------A--------LSZ
          • E→A   • Deletion.

TH-1 EGMVDG 976